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## Purification of very small amount of Tyrosine-phosphorylated protein using proteomic technique

**Do Joon Park** Seoul National University, Korea

Durification of protein is laborious and time-consuming task. Furthermore, purification of very small amount of proteins such as phosphorylated proteins is extremely difficult. A 120-kDa phophoprotein, which is phosphorylated by TSH stimulation, was a target protein which our group tried to purify. Although this phosphoprotein band was reported years ago, this protein is not purified yet thus its role in FRTL-5 cell is yet to be elucidated. Treatment of TSH induces increases of tyrosine phosphorylated protein bands in FRTL-5 cells, although TSH receptor is not a tyrosine kinase itself. Because this TSH effect requires hours of treatment, it seems that TSH receptor does not directly activate tyrosine kinases, but may induce tyrosine kinases or their target proteins. Among these tyrosine phosphorylated proteins, 120-kDa phophoprotein is the most prominent one, whose phosphorylation increases by the treatment of TSH. Analysis of this 120-kDa phosphoprotein by 2D protein gel and anti-phosphotyrosine blot showed that this protein seems to be multiply phosphorylated by TSH, which implies that activity of this protein is tightly regulated by phosphorylation. To purify this 120-kDa phosphoprotein, we combined a series of conventional HPLCs, protein 2D electrophoresis and MALDI-TOF. FRTL-5 cells were harvested after stimulation, and sequential HPLC of DEAE- 5PW, phenyl-5PW and gel filtration chromatography followed by 2D protein electrophoresis could isolate tyrosine phosphorylated spots which were compatible with 120 kDa-phosphoprotein. Results of MALDI-TOF analysis revealed that the peptide sequence of this protein matched perfectly with already known protein, which is important in cell division. This finding suggests that this protein might play some important role in TSH-induced growth of thyrocytes.Combination of proteomic techniques including 2D protein electrophoresis, mass spectrometry as well as Western blot analysis was very effective method to purify very small amount of phosphorylated proteins, which can be used widely to purify very small amount of proteins.

## Biography

Do Joon Park has completed his MD at the age of 25 years from Seoul National University College of Medicine. After completing internship and residency training at the Seoul National University, he went to the U.S. and studied at NIH and Harvard Medical School for 6 years. After coming back to Korea, he has worked at the Seoul National University Hospital. He is the director of the thyroid center, head of the division of endocirinology and metabolism, and the professor of the department of the translational medicine, Seoul National University College of Medicine.

djpark@snu.ac.kr